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# Solid-state fermentation novel process for improving nutritional value of plant feedstuffs

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## Examining use of solid-state fermentation with soy, rapeseed cake, lupine and peas



The test bioreactor consisted of flat trays in a closed chamber with a controlled system for temperature, humidity and processing time.

In response to environmental concerns and increased costs, substantial efforts have been focused on research to reduce or eliminate fishmeal in aquafeeds. Important considerations in the selection and use of alternative protein ingredients for commercial diets include the amounts of essential nutrients available for optimal growth, the digestibility and bioavailability of the nutrients, commercial availability and reasonable cost, and the presence of antinutritional factors.

Enhancement of the nutritional quality of alternative protein ingredients is one of the main strategies for the sustainable development of aquaculture. The efficiency and utilization of alternative plant raw materials could be maximized by means of biotechnological processes aimed at concentrating protein content, improving essential amino acid profiles, reducing the level of antinutritional factors and increasing nutrient digestibility and availability.

## Solid-state fermentation

Among the different biotechnological processes, solid-state fermentation (SSF) has various applications for the bioconversion of plant feedstuffs and production of new products with added value that can be of interest for the aquafeed-manufacturing industry and aquaculture in general.

SSF can be defined in a simple way as a microbial fermentation that occurs in a solid, moist medium. It offers numerous advantages over widely used liquid fermentation, including high-volume production and concentration of the final product with the consequent generation of limited effluent. The positive effects of SSF on the nutritional composition of plant raw materials is essentially based on the capacity of the microorganisms that participate in this bioprocess to synthesize necessary nutrients for their growth from the substrate in which they are reproduced.

Another important advantage of SSF is that the substrates used are often by-products of the agriculture, forestry or food-processing industries and in most cases have limited application in the formulation of aquafeeds for carnivorous species. The use of SSF for upgrading the nutritional characteristics of raw materials has been the aim of numerous studies, and solid fermentation of agro-industrial residues has been proposed as a suitable pretreatment that could improve their use in animal feeding.

The process of SSF can increase, for example, the amount of available protein in the raw materials and reduce some of the antinutritional factors that limit their incorporation into diets for cultured species. However, the implementation of such procedures at industrial scale for the production of aquafeeds still requires considerable research efforts in order to optimize the processes as much as possible.

## Substrate, microorganism selection

In laboratory research, the authors examined the use of solid-state fermentation with soy, rapeseed cake, lupine and peas for the enhancement of their nutritional profiles.

The authors selected various ingredients with different characteristics (substrates with high protein content, starchy substrates and lignocellulosic substrates) as the macromolecular matrix for SSF processing. Ingredients were also considered on the basis of their actual and potential application in aquafeed formulations. Microorganisms commonly used in the food and feed industries were selected for the fermentation essays at laboratory scale: *Lactobacillus plantarum*, *Aspergillus niger*, *A. oryzae*, *Rhizopus oryzae* and *R. oligosporus*.

The variables evaluated considered such fermentation conditions as moisture, pH, fermentation time, surface area/dry mass relationships, microorganism growth, and size and state of inoculum. Changes in the substrates' nutritional profiles for amino acids, fatty acids, minerals, soluble proteins, phosphorus, phytic acid and polysaccharides were also examined.

## Varied results

The results obtained in the laboratory were variable among different substrates and microorganisms, and directly affected by the conditions of the fermentation process. Tables 1 and 2 outline the characteristics for two different microorganisms and substrates used in the fermentation process under the best conditions evaluated to obtain a desirable final product.

The laboratory results served as a basis for the design and construction of a pilot-scale bioreactor capable of replicating and controlling the main variables evaluated in the laboratory to produce up to 30 kg of dry fermented ingredients. The



Preliminary assays using selected ingredients and microorganisms for SSF under laboratory conditions.

bioreactor consisted of flat trays in a closed chamber with a controlled system for temperature, humidity and processing time.

One of the main difficulties observed in other studies conducted on the use of SSF products in aquafeeds was the inability to produce high-volume batches of fermented ingredients at laboratory scale.

The bioreactor was tested and tuned for conducting SFF processes with the selected ingredients using *Aspergillus niger* as a microorganism. Tables 3 to 5 describe the results of proximate analysis and other characteristics obtained during the fermentation process using the pilot bioreactor.

Table 3 shows the proximate compositions of the selected plant ingredients before and after being treated by SSF. The SSF treatment generated significant improvements in some parameters. These results corresponded to the results obtained for SSF production using *A. niger* in the pilot bioreactor. Tables 4 and 5 show additional content values for plant ingredients before and after SSF treatment.

## Hernandez, Characteristics of rapeseed cake, Table 1

	Raw Ingredient	SSF Product	Standard Deviation	Units	Increment

Dry matter		11.16	0.66	%	
Soluble protein	20.15	170.34	9.07	mg/g ss	745%
Soluble phosphorus	0.28	1.51	0.06	mg/g ss	439%
Total sugars	120.90	47.12	5.78	mg/g ss	-61%
Crude fiber	11.06	8.61	0.89	%	-22%
Crude protein	35.26	37.40	2.00	%	6%
NNE	40.49	33.35	0.45	%	-18%
<b>Antinutrients</b>					
Oligosaccharides	25.88	0.89	0.05	mg/g ss	-97%
Phytic acid	0.57	0.17	0.05	mg/g ss	-70%
Total polyphenols	0.89	0.89	0.05	mg/100 g ss	0%
<b>Enzymes</b>					
Proteases		0		UI/g ss	
Cellulases		770.25	50.15	UI/g ss	

Table 1. Characteristics of rapeseed cake fermented with *Lactobacillus plantarum* under optimized laboratory conditions.

## Hernandez, Characteristics of soybean cake, Table 2

	Raw Ingredient	SSF Product	Standard Deviation	Units	Increment
Dry matter		39.30		%	
Soluble protein	104.23	210.13	5.28	mg/g ss	102%
Soluble phosphorus	0.56	39.33	2.74	mg/g ss	6,923%
Total sugars	162.96	18.75	4.04	mg/g ss	-88%
Crude fiber	4.82	3.10	0.19	%	-36%
Crude protein	51.20	65.41	0.53	%	28%
NNE	36.36	21.53	0.45	%	-41%
<b>Antinutrients</b>					
Oligosaccharides	86.19	0		mg/g ss	-100%

Phytic acid	0.73	0.23	0.05	mg/g ss	-68%
Total polyphenols	0.17	0.20	0.01	mg/100 g ss	18%
<b>Enzymes</b>					
Proteases		2,761.62	247.48	UI/g ss	
Cellulases		1,182.20	70.39	UI/g ss	

Table 2. Characteristics of soybean cake fermented with *Aspergillus niger* under optimized laboratory conditions.

## Hernandez, Profiles of plant materials under solid-state fermentation, Table 3

Proximal Profile (%)	Soybean Raw	Soybean SSF	Rapeseed Raw	Rapeseed SSF	Lupin Meal Raw	Lupin Meal SSF	Pea Meal Raw	Pea Meal SSF
Moisture	10.5	5.5	5.4	5.6	4.6	5.58	7.2	6.8
Crude protein	49.1	61.6	35.2	37.4	40.3	52.2	25.5	31.2
Crude fat	2.5	6.7	9.3	7.5	7.2	6.06	1.4	2.3
Total ash	6.4	11.7	5.2	8.6	8.4	9.66	3.4	4.0
Crude fiber	7.7	8.9	11.1	8.6	13.1	9.7	9.3	10.9
NNE	23.8	5.6	33.8	32.3	26.39	16.81	60.4	51.7
Energy (Kcal/100 g)	396	431	424	413	402	418	404	408

Table 3. Profiles of plant materials under solid-state fermentation with *Aspergillum niger* in the pilot bioreactor.

## Hernandez, Other parameters that characterize plant raw materials, Table 4

Parameters	Soybean Raw	Soybean SSF	Rapeseed Raw	Rapeseed SSF	Lupin Meal Raw	Lupin Meal SSF	Pea Meal Raw	Pea Meal SSF
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Soluble protein (mg/g ss)	104.23	210.13	20.15	170.34	29.96	25.96	17.02	75.08
Soluble phosphorus (mg/g ss)	0.56	39.33	0.28	1.51	0.19	0.26	0.32	5.61
Total sugar (mg/g ss)	162.96	18.75	120.9	47.12	30.53	19.21	32.71	40.16

Table 4. Other parameters that characterize plant raw materials after solid-state fermentation using *A. niger*.

## Hernandez, Table 5. Effects of SSF over phytic acid content in treated ingredients, Table 5

Sample	Phytic Acid
Lupin	6.27
Lupin SSF	4.47
Soy	8.79
Soy SSF	<1.77
Pea	7.92
Pea SSF	<1.77
Rapeseed	n.d.
Rapeseed SSF	n.d.

Table 5. Effects of SSF over phytic acid content in treated ingredients.

## Perspectives

The characterization of macro- and micronutrients of plant raw materials treated by SSF in the pilot bioreactor showed it is possible to manipulate the nutritional profile of raw materials through bioconversion processes. The results changed depending on the type of plant substrate used. There was an increment in the amount of protein of all tested ingredients. Positive effects were observed on soluble protein and phosphorus levels, which could have a significant impact on the discharge of these nutrients to the aquatic environment.

The results suggested that SSF could improve the nutritional characteristics of plant protein ingredients and thus decrease or overcome the negative effects of antinutritional factors, making them more promising alternative protein sources for aquafeeds. However, further studies are necessary to determine the direct effects of using SSF products – not only over growth performance and feed utilization, but also nutrient balance and the health status and immune condition of fish.

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